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Biological aspects of *Diopatra* sp. (Onuphidae, Polychaeta) collected from mangrove habitats of Jeruklegi, Cilacap Regency

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ABSTRACT

Diopatra species are members of the family Onuphidae (Polychaeta, Annelida) that are widely used as fishing bait. These tubicolous worms are common in particular mangrove areas of Cilacap. However, biological aspects of *Diopatra* sp. inhabiting the areas have not been studied. In the present work, we investigated several biological aspects of the worms, including the segment number, the body weight and the sex ratio, based on *Diopatra* samples collected from four mangrove habitats of Jeruklegi Village, Cilacap Regency, between June and July 2021. The results show that the average numbers of segments of *Diopatra* sp. obtained ranged from 195 to 216 segments, whereas the average body weights ranged from 2.03 to 3.12 g, and the average sex ratios ranged from 0.5 to 1.3. In general, the biological aspects of *Diopatra* sp. between sampling sites were relatively similar; this can be attributed to the similar environmental conditions at all sampling sites, including salinity (25–32 ppt), water temperature (30–32 ^{o}C), dissolved oxygen (6.4–8.8 ppm) and pH (7). While *Diopatra* worm farming is necessary so that local people do not rely on natural catch, a further taxonomic study is required to reveal the identity of this economically important local species.

Introduction

Diopatra Audouin and Milne Edwards, 1833, is one genus of the family Onuphidae with 67 described species worldwide (WoRMS Editorial Board, 2022). Members of this genus are characterized as having spiral branchiae, serrated limbate chaetae, and styles of palps and antennae covered with sensory papillae (Budaeva and Fauchald, 2011). These tubicolous worms occur in temperate and tropical coastal ecosystems worldwide, typically inhabiting protected intertidal and subtidal flats of muddy sand or sandy mud (Berke, 2022).

Diopatra species possess both ecological and economic importance. Ecologically, the tube structure of *Diopatra* stabilizes sediments, facilitating the attachment of some algal species and therefore provides protection from predators for many marine organisms (e.g. Freitas *et al.*, 2015). Some *Diopatra* species are also well-known for their use as fishing bait. *Diopatra neapolitana* Delle Chiaje, 1841, for instance, is a European species that are massively harvested – about 4.3 million individuals of the species may be harvested each year in Portugal's Canal de Mira (Cunha *et al.* 2005). *Diopatra neapolitana* was historically cosidered cosmopolitan and reported to occur in many places of the world such as the Mediterranean, Africa and Southeast Asia (see the most recent review of the ecological aspects of *Diopatra* species by Berke, 2022). However, such cosmopolitan distribution of polychaete species are now known to be quite unlikely (Hutchings and Kupriyanova, 2018) that the worms previously identified as *D. neopolitana* from geographic regions far away for the type locality are most likely of different species.

Diopatra sp. is commonly found in particular mangrove areas of Cilacap, and is widely used as

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fishing bait by the locals. However, the biological information about the species is still lacking compared to that of another local species, i.e. *Nereis* sp. (family Nereididae). The potential of *Nereis* sp. collected from some aquaculture areas in Randusanga and Pengaradan, Brebes Regency, as natural shrimp feed has been studied by Yuwono *et al.* (1997), Yuwono (2005) and Siregar and Yuwono (2005), whereas Abida (2012) investigated the nutritional contents of the species collected from Kwanyar Beach, Bangkalan Regency. Some biological aspects of *Nereis* sp. collected from Jeruklegi, Cilacap Regency, and their potential as natural shrimp feed have also been studied by Wibowo *et al.* (2018; 2020).

An initial study regarding biological aspects of *Diopatra* sp. inhabiting mangrove areas in Cilacap is of importance as the basis of knowledge for the species culture (the local species is most likely to have the potential to be used as natural feed for shrimp broodstock). This is because the removal of *Diopatra* sp. from their natural habitat is generally considered unsustainable for the environment as it contributes to the depletion of natural resources (e.g. Pires *et al.*, 2012); polychaete aquaculture is hence the solution. In the present work, we aimed to study several biological aspects of *Diopatra* sp. inhabiting mangrove habitats of Jeruklegi, Cilacap Regency.

Materials and Methods

Location and time of research

This research was conducted in mangrove habitats of Jeruklegi Village, Cilacap Regency, and the Experimental Laboratory of the Faculty of Biology, Jenderal Soedirman University, Purwokerto, Banyumas Regency. *Diopatra* samples were collected from four sampling sites (see Figure 1 and Table 1) three times every fortnight, from June to July 2021.

Materials

The materials used were sample boxes, plastic bags, stereo and compound microscopes, object glass, cover glass, cavity slides, capillary tubes, tweezers, petri dishes, and *Diopatra* samples collected from the four mangrove habitats of Jeruklegi, Cilacap.

Research design

The present research used purposive sampling to specifically obtain *Diopatra* samples from the animals' habitat. The animals were collected from four mangrove habitats, each of which with five sampling points. At each point, the animals were collected from a quadrat with a size of 1 x 1 m^2.

Figure 1. Map of the sampling sites. Map of Indonesia (top-left corner) was created using *SimpleMappr* (https://www.simplemappr.net). The other two maps were created using *Google Maps* with some edits.

Table 1. Sampling sites and the coordinates.

Sampling site	Coordinate
1	7°39'48.5"S, 109°02'00.5"E
2	7°39'53.4"S, 109°02'01.7"E
3	7°39'58.5"S, 109°02'04.0"E
4	7°40'02.6"S, 109°02'03.2"E

Sample collection

Diopatra samples were collected from the mangrove substrate (muddy sediment) using a hand shovel during a low tide (the presence of the animals can be observed by looking at the tip of the animals' tube showing up on the sediment surface). At each sampling point, living individuals of *Diopatra* were placed in a tray and labeled according to the sampling site. The samples obtained were then transported to the laboratory for further analyses.

Environmental parameter measurement

At each sampling site, several environmental parameters, i.e. water temperature, salinity, pH and dissolved oxygen, were measured using a thermometer, a hand refractometer, a universal pH indicator paper and the winkler method, respectively.

Laboratory work

For the identification purpose, an adult worm was selected and fixed using formalin 4% for about 24 hours. The worm was then rinsed using tap water and transferred into a bottle containing alcohol 70%. Afterwards, the animal was identified under both stereo and compound microscopes using the identification keys created by Fauchald (1977) and Budaeva and Fauchald (2011). This identification step was necessary to ensure if the worms obtained from the field are *Diopatra* sp.

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Furthermore, the number of body segments of living *Diopatra* sp. was counted, and the animals' bodyweight was weighed using an analytical balance with an accuracy of 0.01 g. The animals' sex was determined by looking at the gametes extracted from the coelom cavity by dissecting the animals' body. The body was then gently pressed on an object glass to release the gametes. A drop of salty water was then added, and the gametes were observed under a compound microscope using a magnification of 100x. After the animals' sex was determined, the sex ratio was calculated using the formula of Belal (2012), i.e. sex ratio = number of females/number of males.

Data analysis

The data resulted from this study were compared between sampling sites using the non-parametric Kruskal-Wallis test as the data were found to be not normally distributed. In addition, the Spearman correlation analysis between the number of segments and the body weight of *Diopatra* sp. was also performed. Both analyses were done using R version 4.2.2.

Figure 2. Anterior morphology of *Diopatra* sp. collected from mangrove habitats of Jeruklegi, Cilacap (the specimen is 10.5 cm long and 1 cm width). Note: an = antenna, bra = branchiae, fl = frontal lip, pa = palp, par = parapodium, pc = peristomial cirrus.

Results

All the samples identified agree well with the description of the genus *Diopatra* by Budaeva and Fauchald (2011). The worms obtained had spiral branchiae, serrated limbate chaetae and styles of palps and antennae covered with sensory papillae (Figure 2). The individual numbers of *Diopatra* sp. collected in the present study ranged from 17 to 31 individuals as presented in Table 2.

The *Diopatra* worms obtained had a number of segments ranging from 84 to 478, with an average number of segments ranging from 195 to 216. However, the statistical analysis shows that the number of segments of *Diopatra* sp. between sampling sites was not significantly different ($p > 0.05$; without sex distinction) as presented in Table 2.

The bodyweight of *Diopatra* sp. ranged from 0.34 to 6.52 g, with an average body weight ranging from 2.03 to 3.12 g. The statistical analysis shows that the bodyweight of *Diopatra* sp. between sampling sites was not significantly different ($p > 0.05$) as presented in Table 2.

Additionally, the Spearman correlation analysis strongly suggests that the number of segments of *Diopatra* sp was positively correlated with their body weight ($R = 0.93$; $p < 0.05$) as presented in Figure 3.

The ratio of females to males of *Diopatra* sp. ranged from 0.5 to 1.3. The statistical analysis shows that the sex ratio of the animals between stations was not significantly different ($p > 0.05$) as presented in Table 3.

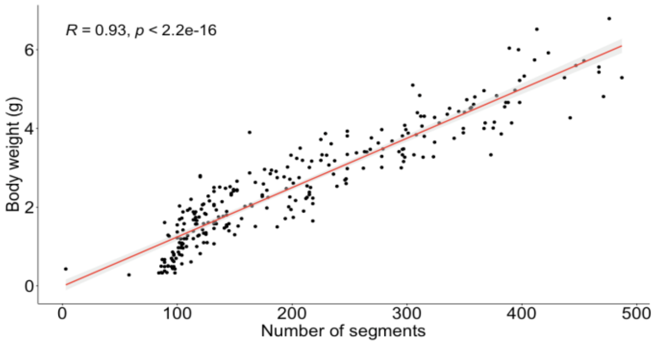

Figure 3. Spearman correlation analysis between the number of segments and the body weight of *Diopatra* sp. Red-solid line is the linear regression line. Light grey shading is the standard errors. *R* is the Spearman's correlation coefficient. *p* value (2.2e-16) indicates that the correlation between the two variables was highly significant.

Table 2. The individual numbers, the segment numbers and the body weights of *Diopatra* sp. obtained in the present study.

Sampling site	Individual number range	The average number of individuals	Segment number range	The average number of segments*	Body weight range (g)	Average body weight (g)*
1	21–27	21.00 ± 5.57	183–213	195.24 ± 16.09[a]	2.03–3.03	2.49 ± 0.50[a]
2	17–30	22.67 ± 6.66	173–250	205.90 ± 39.82[a]	2.33–2.49	2.62 ± 0.44[a]
3	18–30	23.33 ± 6.11	152–231	195.10 ± 39.73[a]	2.41–3.12	2.86 ± 0.40[a]
4	19–31	23.33 ± 6.66	187–262	216.19 ± 40.29[a]	2.40–3.00	2.57 ± 0.38[a]

*Note: Numbers followed by the same letter suggest no significant difference ($p > 0.05$)

Diopatra worms collected from mangrove habitats of Jeruklegi, Cilacap, were mostly found in immature conditions that the sex was not distinguishable as presented in Table 4.

The environmental conditions at the four sampling sites, which include water salinity, temperature, dissolved oxygen and pH, were found to be relatively similar as presented in Table 5.

Table 3. The sex ratios of *Diopatra* sp. obtained in the present study.

Sampling site	Range of sex ratio	Average sex ratio*
1	0.75–1	0.92 ± 0.14[a]
2	0.5–1	0.83 ± 0.25[a]
3	0.5–1.3	0.93 ± 0.40[a]
4	0.8–1.17	0.99 ± 0.19[a]

*Note: Numbers followed by the same letter suggest no significant difference ($p > 0.05$)

Table 4. The average numbers of sexes of *Diopatra* sp. obtained in the present study.

Sampling site	Male	Female	Immature
1	5.6	6	9.3
2	6.6	4.6	11.3
3	5.3	4.3	13.6
4	5.6	5.6	12

Table 5. Environmental data at each sampling site.

Sampling site	Salinity (ppt)	Temperature (°C)	Dissolved O_2 (ppm)	pH
1	27–30	30–32	6.8–8.8	7
2	25–32	30–32	6.4–7.2	7
3	27–31	30–32	6.8–8.8	7
4	25–31	30–32	6.4–8.8	7

Discussion

The results show that the average number of *Diopatra* sp. obtained between mangrove habitats in Jeruklegi, Cilacap, was not significantly different ($p > 0.05$; Table 2). This similar number of catch is mainly due to similar environmental conditions at all sampling sites (Table 5). The polychaete diversity of the Segara Anakan mangrove areas has been investigated by Pamungkas (2013), but interestingly *Diopatra* sp., including other members of the family Onuphidae, was not recorded in the study even though some of the samples were collected from mangrove habitats near Jeruklegi Village. This is most likely because Pamungkas (2013) collected his samples near an industrial area around Donan Creek, which was reported to be contaminated mostly by polycyclic aromatic compounds (Dsikowitzky *et al.*, 2011), whereas the samples of the present study were obtained from mangrove habitats that are far away from the industrial area of Cilacap. These findings thus suggest that *Diopatra* sp. may be used as a biondicator of the health of the mangrove habitats in the study area.

We further found that the average number of segments of *Diopatra* sp. ranged from 195 to 216; the animals' segment number was not significantly different between sampling sites (Table 2). In addition, the preserved specimen (Figure 2) measured 10.5 cm in length and 1 cm in width. The segment number range of *Diopatra* sp. is similar to that of *Nereis* sp. (Nereididae) collected from the same mangrove area, i.e. 178–220 segments (Wibowo *et al.* 2018). Nevertheless, the size of *Diopatra* sp. in the present work is generally smaller than that of European species. For example, Cunha *et al.* (2005) reported that *D. neapolitana* collected from Portugal's Canal de Mira (Ria de Aveiro estuary) had a body length of 15–50 cm, whereas the same species collected from the same area was reported to have a body length of 2.4–72.5 cm by Pires *et al.* (2012). Additionally, the biggest *D. neapolitana* collected from the Villaviciosa estuary, Asturias, northern Spain, measured 80 cm in length with 300 segments (Arias *et al.*, 2016).

The similar number of segments in *Diopatra* sp. in this study implies the similar bodyweight of the

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species (Table 2). The increase in the number of segments and the body weight – both variables had a strong positive correlation (Figure 3) – indicates the animals' growth, and can be affected by several factors such as salinity and feed. A research by Wibowo *et al.* (2019) suggested that the polychaete worms *Nereis* sp. fed by animal protein had a greater number of segments and weight compared to the worms fed by vegetable protein. The number of segments and the body length of a polychaete increase as the species grows, and stop when it enters the adult phase (Junardi, 2018).

The number of segments as well as the body length and weight of *Diopatra* sp. are apparently not sex-dependent. This is supported by our other findings that the ratio of female to male *Diopatra* worms between sampling sites (including the immature worms) was not significantly different (Table 3 and 4). The sex ratio of *Diopatra* worms obtained in the present work is relatively similar to that of the Portuguese *D. neapolitana* collected by Pires *et al.* (2012), where the ratio of males to females was 1 : 1, especially in April–September. During our study, we also observed that the morphology of both male and female *Diopatra* worms was generally similar and not distinguishable (Figure 2) until they were sexually mature and ready to reproduce. In this stage, the body of male and female individuals turned creamy and dark green, respectively. These findings are in line with what Pires *et al.* (2012) pointed out that there was no morphological difference between male and female *D. neapolitana*, but when the animals were ready to reproduce, the males turned creamy whereas the females turned greenish because of the presence of the gametes in the body coelom.

In terms of environmental parameters, we found that the salinity level between sampling sites was relatively similar ($p > 0.05$); the salinity level ranged from 25 to 32 ppt (Table 5). This relatively high range of salinity is geographically due to the small amount of fresh water (mostly from the rainfall) that flows into Donan Creek in the eastern part of the Segara Anakan Lagoon (this is different from the western part of the lagoon which receives the large amount of fresh water from both Cibeureum and Citanduy Rivers), resulting in a higher diversity and a bigger size of polychaetes around the creek as the animals generally prefer habitats with a higher salinity (Pamungkas, 2013). The salinity range in the four mangrove habitats of Jeruklegi, Cilacap, was still within the tolerable range for *Diopatra* species, i.e. 15–40 ppt (Hakkim, 1975), although studies by Freitas *et al.* (2015) and Pires *et al.* (2015) suggested that the regenerative capacity of *D. neapolitana* was optimum at salinities 28 and 35 ppt. Both studies also revealed that both lower and higher salinities (i.e. 21 and 42 ppt, respectively) resulted in a lower regenerative capacity of the species – more days were needed to completely regenerate the missing body region.

The water temperature at all sampling sites was relatively similar, i.e. ranging from 30 to 32°C (Table 5). Our findings hence indicate that *Diopatra* worms in the present study live in the intertidal habitats with a considerably higher water temperature range than the temperate *D. neapolitana*; the latter species inhabits intertidal areas with a water temperature ranging between 17 and 21°C (Vaz *et al.*, 2005; Pires *et al.*, 2015). Pires *et al.* (2015) explained that the regenerative capacity of *D. neapolitana* increased with the increase of water temperature – the individuals exposed to a higher water temperature (24°C) were found to regenerate faster than those exposed to lower temperatures. The optimum water temperature to stimulate the regeneration process of *Diopatra* sp. is nonetheless still unknown; a subsequent study is thus required.

The dissolved oxygen between sampling sites was not considerably different ($p > 0.05$); the level of dissolved oxygen ranged between 6.40 and 8.80 ppm (Table 5). This result is in accordance with the study by Romadhoni and Aunurohim (2013), suggesting that polychaete worms in the mangrove area of Kali Lamong, Gresik, could survive the environmental condition with a dissolved oxygen level ranging from 4.28 to 7.51 ppm. Besides, many polychaete species living in mangrove habitats, including *Diopatra* sp., possess branchiae (see Figure 2) as a respiratory feature that helps the animals to breathe.

The pH value at all sampling sites was the same, i.e. 7 (Table 5). This level of pH is more or less still favorable for the growth of *Diopatra* worms in Jeruklegi, Cilacap, despite the fact that its level is slightly lower than the optimum pH needed by *D. neapolitana* to live, i.e. between 7.5 and 7.8; the species exposed to a lower level of pH demonstrated a considerably lower capacity to regenerate their body (Pires *et al.*, 2015). That *Diopatra* sp. in the present study survived a slightly lower level of pH may be because the animals belong to a different species that is more tolerant towards a lower pH; a subsequent identification to the species level is therefore necessary.

Overall, we found that all the biological aspects of *Diopatra* sp., including the number of segments, the body weight and the sex ratio, were not significantly different between sampling sites ($p > 0.05$). This is mostly related to the similar environmental conditions at the four sampling sites (Table 7). Changes in environmental conditions, especially pH, salinity and temperature, were reported to affect the regeneration ability of *Diopatra*; unfavorable ecological conditions will hinder the regeneration process of the species (e.g. Freitas *et al.*, 2015; Pires *et al.* 2015). However, the environmental conditions of mangrove habitats of Jeruklegi, Cilacap Regency, were generally found to be favorable for the growth of *Diopatra* sp.

Conclusion

Diopatra worms collected from mangrove habitats of Jeruklegi, Cilacap Regency, had the average number of body segments ranging from 195 to 216, the average body weight ranging from 2.03 to 3.12 g, and the average sex ratio ranging from 0.5 to 1.3. All the biological aspects of *Diopatra* sp. between sampling sites were found to be relatively similar; this can be attributed to the similar environmental conditions at all sampling sites, including salinity (25–32 ppt), water temperature (30–32^{o}C), dissolved oxygen (6.4–8.8 ppm) and pH (7).

Suggestions for further research include more seasonal sampling, comprehensive biological data and analyses (e.g. a proximate analysis to reveal the macronutrient contents of the animals), and environmental data (e.g. the measurement of organic matter). In the future, *Diopatra* worm farming may be necessary so that the locals do not rely on digging activities to obtain the worms, which can endanger the sustainability of the species. Lastly, a further taxonomic study is required to reveal the identity of *Diopatra* sp. inhabiting mangrove habitat of Jeruklegi, Cilacap.

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